



## Original article

## Pollination ecology of a plant in its native and introduced areas

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## ARTICLE INFO

## Article history:

Received 13 March 2013

Accepted 10 January 2014

Available online 11 February 2014

## Keywords:

Biogeography

Biological invasions

Fruit set

*Hedysarum coronarium*

Mutualistic interactions

Pollen deposition

Pollinators

## ABSTRACT

Entomophilous and obligate out-crossing non-native plants need to become well integrated in the resident plant–pollinator network to set seeds and become established. However, it is largely unknown how pollination patterns differ between native ranges and those where plants have been introduced.

We compared the identity, abundance and visitation rates of pollinators, insect pollen loads, pollen deposition on stigmas, and fruit and seed sets of *Hedysarum coronarium*, an entomophilous short lived N-fixing perennial, in populations from native and introduced ranges in Spain (South of mainland Spain and Menorca Island, respectively).

In both areas, *Hedysarum* was visited by a similar number of species, mainly hymenopterans; seven species were common between native and introduced areas. However, pollinator richness, abundance, and visits per flower were greater in the native than in the introduced range, as were fruit and seed sets. *Hedysarum* pollen loads on stigmas and on *Apis mellifera*, the most common pollinator, did not differ between areas. Lower abundance of pollinators might be causing lower visitation rates, and to some extent reducing *Hedysarum* fruit and seed sets in the introduced area.

Our biogeographical approach shows that integration of a non-native plant in a resident pollinator network does not prevent pollen limitation in the introduced area. Therefore, despite being necessary, pollination mutualistic relationships might not be the key for non-native plant establishment success in the introduced area.

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## 1. Introduction

The success of many non-native plants in the range where introduced depends on the mutualistic relationships they establish with the resident biota (Richardson et al., 2000). For instance, entomophilous and obligate out-crossing non-native plant species require resident pollinators in order to reproduce and to eventually invade (Parker, 1997; Chittka and Schürkens, 2001; Vanparys et al., 2008; Rodger et al., 2010; Goodell et al., 2010; Gross et al., 2010). However, most research on the pollination of non-native plant species has focussed on their impact on the pollination and subsequent reproductive success of co-flowering native species (Traveset and Richardson, 2006; Bjerknes et al., 2007), rather than on the role of pollination in facilitating or constraining their naturalization and/or invasion (but see Parker, 1997; Parker and Haubensak, 2002; Stout et al., 2002; Simpson et al., 2005; Gross et al., 2010; Rodger et al., 2010).

Generalization in pollination is more often the rule than the exception (Jordano, 1987; Waser et al., 1996), enabling non-native plants to quickly integrate into resident plant–pollinator networks (Memmott and Waser, 2002; Vilà et al., 2009). In many cases, super-generalist pollinators such as the honeybee *Apis mellifera* and bumblebees *Bombus* spp., which have been introduced worldwide and often massively, play a key role in such integration (Stout et al., 2002; Simpson et al., 2005; Jesse et al., 2006; Gross et al., 2010).

It is not only non-native plants with generalist pollination systems that integrate into resident plant–pollinator communities, but specialist species can also be integrated in different ways. Some may find specialist pollinators if these have wide distribution ranges or have also been introduced there (i.e. “alien complexes” *sensu* Olesen et al., 2002). Other non-native plants may generalize their specialist pollination behaviour, as in the case of *Fuchsia magellanica*, which in its native range in South America is mainly visited by a hummingbird (*Sephanoides galericus*) (Traveset et al., 1998) while in its area of introduction in Britain is visited by several generalist insects (Valentine, 1977). Even self-pollinated plant species may be included in resident plant–pollinator communities, promoting their naturalization and eventual invasion

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through increased out-crossing and seed sets, if autonomous self-pollination does not result in the fertilization of all ovules (Aizen and Harder, 2007).

In addition to non-native plants being integrated into the resident plant–pollinator community, their pollination success requires pollinator visits to be efficient in terms of quantity and quality of pollen loads transported among conspecific plant individuals (Feinsinger, 1987; Aizen and Harder, 2007; Mitchell et al., 2009). Pollination efficiency is context dependent (Potts et al., 2001; Ne'eman et al., 2010). Moreover, subsequent reproductive success of non-native plants also depends on plant variables (requirement of a minimum threshold of pollen deposition for fruit and seed production, etc.) (Ne'eman et al., 2010). Therefore, in some cases, as it has been observed for the invasive *Lonicera maackii*, high visitation rates do not prevent pollen limitation (Goodell et al., 2010); while in other cases visitation rates can constitute a good surrogate of reproductive success (Parker, 1997; Vázquez et al., 2005). Furthermore, pollinator communities show high inter-annual variability (Roubik, 2001; Petanidou et al., 2008). Therefore, although this is rarely done (but see Parker, 1997; Brown et al., 2002; Moragues and Traveset, 2005; Jesse et al., 2006; Dietzsch et al., 2011), studies should contemplate more than one season in order to attribute the invasion process of a plant species with the relationships it establishes with the resident pollinator community (Petanidou et al., 2008).

Most studies on the role of insect mediated pollination of introduced plants have been conducted solely in the introduced range, often with little knowledge of the pollination ecology in the native range. A biogeographical approach comparing native and introduced areas would help to disentangle the processes that enable non-natives to succeed in their new ranges (Hierro et al., 2005; van Kleunen et al., 2010). Information on the pollination ecology and reproductive success in native and introduced areas of some plant species is available from different studies; e.g. *Cytisus scoparius* (see Parker, 1997; Parker and Haubensak, 2002; Suzuki, 2003; Simpson et al., 2005; Galloni et al., 2008; Paynter et al., 2010) and *Nicotiana glauca* (compiled in Ollerton et al., 2012). But, to our knowledge, only the pollination interactions of *Rhododendron ponticum* have been studied from this biogeographical perspective by following a standard field sampling protocols both in the native and introduced areas (Stout et al., 2006).

In this study we apply this biogeographical approach to the pollination ecology and reproductive success of an entomophilous plant species whose native and introduced areas have a close regional proximity. Our main questions are: a) Do the identity of pollinators and generalization degree of plant populations differ between native and introduced areas? b) Do pollinator richness, abundance, and visitation rates differ between the two areas? c) Do pollen loads on the main pollinator species and on stigmas differ between the two areas? and finally d) Do fruit and seed sets differ between areas? Our hypothesis is that an entomophilous non-native plant species which has become naturalized in a new area might have similar pollination patterns as in the native area. We expect resident pollinators to provide non-native plants a pollination service preventing pollen limitation and allowing for similar seed sets than in their native area.

## 2. Materials and methods

### 2.1. Study species

*Hedysarum coronarium* L. (Fabaceae; hereafter *Hedysarum*) is a short-lived N-fixing and sexually reproduced perennial (Sulas et al., 2000; Bullitta et al., 2000) that can reach 1.5 m tall when erect in growth habit, but can also be prostrate (Montes, 1993/94;

Bustamante et al., 1998). Its inflorescences are racemes with up to 30 pink flowers with 1 cm long corollas, rich in pollen and nectar that bloom during April and May. Its flowers need to be tripped and are pollinated mainly by bees (Louati-Namouchi et al., 2000a,b; Satta et al., 2000) and are self-compatible but present high out-crossing rates (Yagoubi and Chriki, 2000; Louati-Namouchi et al., 2000a). *Hedysarum* is native of the south-western Mediterranean basin (Talavera et al., 1988), where it grows from sea level to low frost-free altitudes (Gutiérrez, 1982). It has been introduced as a forage plant in other semiarid regions of the Mediterranean basin because of its high feed value for cattle (Yagoubi and Chriki, 2000). It is also used for erosion control, re-vegetation, and high-quality honey production (Flores et al., 1997; Satta et al., 2000). Currently, whether naturally or due to human intervention, it grows in many Mediterranean basin countries, from Turkey to Spain (Flores et al., 1997).

### 2.2. Study sites

The study was conducted in two areas of Spain. The native area was located in the province of Cádiz, S Spain, while the introduced area was the NE of Menorca, the northernmost of the Balearic Islands (Fig. 1). These areas have a close regional proximity and share a Mediterranean climate with similar average monthly temperatures around 17 °C, and an average annual precipitation of 600 mm (AEMET). We are aware that, as the introduced area is an island, description of patterns of pollination between native mainland areas and introduced insular areas cannot disentangle nativity from insular differences. However, in insular areas the introduction and potential invasion of species from mainland is a highly common phenomenon (Kueffer et al., 2010) that deserves exploration even if causality cannot be inferred. Furthermore, the close geographic proximity between the native and the introduced allows for comparing highly similar ecological settings (i.e. climate, vegetation type, species assemblages, landscape configuration), reducing the influence of major confounding factors that preclude any causality.

In Menorca, *Hedysarum* was introduced between the end of the 18th and the beginning of the 19th centuries (Ortells and Campos, 1983). Since 1860 it has been used in a traditional cyclical agro-farming system, which consists of growing crops of *Hedysarum* for two consecutive years, followed by cereal cropping the third year and leaving the land fallow in the fourth year (Bustamante et al., 2007). To some extent, this traditional system is still used on the island with minor modifications. Today, *Hedysarum* has escaped from crops and is well established (i.e. naturalized *sensu* Pysek et al., 2004) in natural and semi-natural areas (Fraga et al., 2004) such as ditches, old-fields, field edges, and ruderal areas.

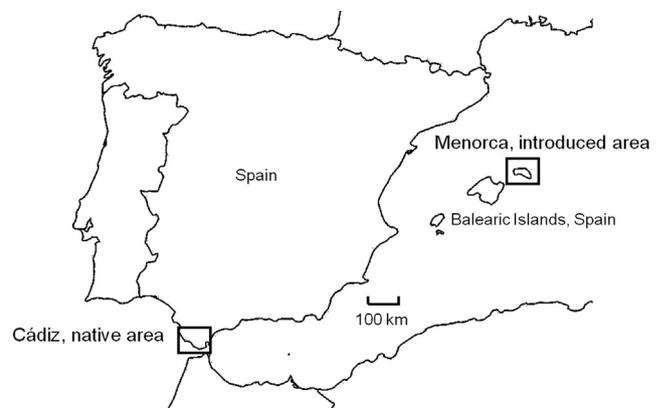


Fig. 1. Location of the study areas.

In each study area, we selected four 400 m<sup>2</sup> plots of early successional shrublands dominated by *Olea europaea* ssp. *sylvestris* and *Pistacea lentiscus* with a rich herbaceous understorey located in similar landscape types (i.e. dispersed human settlements close to coastal areas). Managed honeybee hives were absent within the 500 m radius around all study plots.

*Hedysarum* cover (mean ± SE) was similar in both areas (Table A.1, Appendix A): 49.53 ± 7.46% in Cádiz (native area) and 47.69 ± 13.41% in Menorca (introduced area) plots (Wilcoxon test statistic = 10, *P* = 0.69).

In the introduced area the average (±SE) distance between plots was 9247.00 ± 3522.21 m, but distances were shorter in the native area: 632.55 ± 100.30 m (Table A.1). In the native area in S Spain, many communities with *Hedysarum* were not suitable for the study because they were grazed by cattle, were located in forbidden military precincts, were located inland at a distance to the coast farther than selected plots in Menorca, or were smaller than the established 400 m<sup>2</sup>. Considering the mentioned limitations, we sought to maximize plot distances so that plots were as independent as possible, but also to have similar *Hedysarum* population sizes, vegetation and landscape structure as in the introduced area.

Despite other pollinator studies having also used distances between plant populations similar to ours (see Dohzono et al., 2008; Yang et al., 2011a; King and Sargent, 2012), we are aware that honeybee *A. mellifera* and some bumblebees *Bombus* ssp. can embrace larger maximum foraging ranges (Osborne et al., 2008; Bommarco et al., 2010). However, they often limit their foraging distances when diverse and abundant flower resources are available at the local scale (Johnson et al., 2003; Greenleaf et al., 2007; Wolf and Moritz, 2008). In our plots, more than 15 plant species (Montero-Castaño personal observation) were in bloom simultaneously with *Hedysarum*, providing abundant and diverse floral resources. In addition, the maximum foraging distances of solitary bees range from 150 to 600 m (Gathmann and Tscharrntke, 2002), and coleopterans in general are highly sedentary (Mawdsley and Sithole, 2009). Therefore we consider our study plots to be suitable for the objectives of the study.

### 2.3. Pollination censuses

We conducted pollination censuses on *Hedysarum* simultaneously in both study areas and during two consecutive seasons: spring 2009 and 2010. Weather conditions in both study years fell into the average ranges for the study areas (AEMET).

Pollination censuses were performed during sunny, warm (≥17 °C) and non-windy days, from 10 a.m. to 6 p.m. Plots within each study area were alternatively surveyed. Within each plot, we randomly selected patches of *Hedysarum* with different sizes and distances to closest conspecifics, because flower spatial distribution and abundance can affect pollination patterns (Mustajarvi et al., 2001; Somanathan and Borges, 2001). To avoid any bias, selected patches for observation ranged from small and isolated to large and close to conspecifics. The size of patches finally ranged from 14 to 563 flowers under observation. We observed pollinators visiting plants for 15 min periods. During each observation period, we counted the number of flowers observed, the number and identity of pollinators, and the number of visits of each pollinator species. A visitor was considered a pollinator when it tripped and entered a flower and touched the sexual parts of the plant. The species that could not be identified in the field were recorded as distinct morphospecies and caught for later identification by specialists. Voucher specimens are deposited at EBD-CSIC.

As abundance and evenness of pollinators was not expected to be the same in each plot, the number of censuses differed in each plot, establishing a compromise between sampling effort and

quality of the data collected. We considered a plot to be properly surveyed when, according to its rarefaction curve (Appendix B), we found no new visitor species after three or more observation periods. Nevertheless, to overcome any difference in sampling effort, in each plot we extrapolated the expected *Hedysarum* pollinator generalization degree (i.e. the expected number of visitor species) with the first-order Jackknife species-richness estimator. We considered this estimator to be the most suitable one for our data because non-parametric estimators are less sensitive to unevenness of species incidence, and in general perform better than the ones based on species–accumulation curves or on species–area relationships (Brose et al., 2003; Hortal et al., 2006). In addition, the first-order Jackknife estimator has been demonstrated to perform adequately for non-biased, precise, and accurate estimations when sampling coverages differ, and the grain of the measures is small and constant among all the plots (Hortal et al., 2006), as was the case in this study.

We estimated *Hedysarum* degree of generalization by the first-order Jackknife for each study area and year separately and for both years pooled. We compared *Hedysarum* generalization degree between native and introduced areas by looking at the 95% confidence of the generalization degree estimates averaged from 100 randomizations.

We compared the number of pollinator species, number of individuals and number of visits (hereafter richness, abundance, and visitation rates, respectively) between native and introduced areas after controlling for the number of observed flowers in each observation period. We explored the differences in these response variables within the R statistical computing environment (R Development Core Team, 2011 <http://www.R-project.org/>) by building generalized mixed models (*lme4* library). Area (native/introduced) was included in the model as a fixed effect, the logarithm of the number of observed flowers as offset, plot, and year as random effects and log as link function of the Poisson family. We also explored differences in the number of visits per individual pollinator for the total pool of pollinator species and for the most common pollinator species (honeybee) by building linear models with area (native/introduced) as fixed effect. Values are given as mean ± SE, unless otherwise mentioned.

### 2.4. Pollen loads on honeybee

As the honeybee was the most common *Hedysarum* visitor species, we assessed its efficiency in carrying *Hedysarum* pollen and whether pollen dispersal differed between areas. Pollinators, when foraging, seek to optimize their food intake (Armbruster and Herzig, 1984), adapting their foraging behaviour to the abundance, density and quality of available floral resources (Mustajarvi et al., 2001). Flowering communities were not exactly the same between the native and the introduced areas, either in terms of plant species identity or their relative abundances. In addition, pollinator visits are not equally efficient in terms of pollen removal and transport, depending on the plant species visited, on their spatial distribution (Ne'eman et al., 2010), etc. Therefore, we could expect honeybees to carry different percentages of heterospecific pollen between areas.

During the flowering peak of 2009 field season (mid-April), 10–15 specimens per plot were captured just after a visit to a *Hedysarum* flower. We preserved each captured specimen individually in a paper bag inside a plastic vial with a piece of cotton soaked with ethyl acetate (Forup and Memmott, 2005; Gibson et al., 2006; Lopezaraiza-Mikel et al., 2007). We obtained two pollen samples per specimen by rubbing two small cubes (0.3 × 0.3 mm<sup>2</sup>) of fuxine-stained glycerine jelly (Beattie, 1971) on the ventral and dorsal parts, respectively, of each bee body. The samples were

mounted on microscope slides for examination. Pollen identification was based on a reference pollen collection of the flowering plant species in the study areas. However, as the frequency of pollen from other species was very low, we distinguished only two categories: *Hedysarum* and heterospecific pollen. Percentages of these two pollen categories were quantified by combing the entire slide and counting all the pollen grains in every two 200× magnification microscope fields (Bartomeus et al., 2008).

We explored differences in the percentages of *Hedysarum* and heterospecific pollen loads by linear mixed models. The response variables were logit-transformed according to Warton and Hui (2011). The area (native/introduced) and body part (dorsal/ventral) were included in the models as fixed effects, and individual nested in plot as a random effect.

### 2.5. Pollen deposition on stigmas

We also examined the pollen deposited on *Hedysarum* stigmas in the two study areas. In each plot, we collected 15 *Hedysarum* flowers of different individuals. To be sure that they were not virgin, we collected them immediately after being visited by a honeybee. We kept flowers in separate paper bags. Later in the laboratory, in order to avoid self-pollen deposition, we accessed the styles by removing the wings and keel petals from the base of the corolla with forceps. Extracted styles were immersed in a drop of melted fuxine-stained glycerine jelly on a microscope slide. As before, for the identification and quantification of pollen loads, slides were examined at 200× magnification. We considered only the pollen adhering to the stigma hairs. As for pollen loads, all heterospecific pollen was grouped in a single category because of the low incidence. Accurate pollen counts were not always feasible because pollen grains were sometimes clumped or masked by stigma tissue. Therefore, as in Bartomeus et al. (2008), our analysis of pollen abundance on stigmas was semi-quantitative. We established six abundance categories: absent, present (only one grain), low ( $\leq 25\%$  of the grains), medium ( $25\% < > 75\%$ ), high ( $\geq 75\%$ ), and sole (100%). For each collected stigma, one abundance category was assigned for *Hedysarum* pollen and another for heterospecific pollen. For both *Hedysarum* and heterospecific pollen depositions, we performed Chi-square tests for each category in order to assess any differences between the native and the introduced areas.

### 2.6. Fruit and seed sets

In each plot, during the flowering peak of 2010 (mid-April) we randomly singled out 18–20 *Hedysarum* plants and, in each, marked 3 flowers from 3 different inflorescences to which we randomly assigned one of the following treatments: a) open pollination: flowers were not manipulated; b) autonomous self pollination: inflorescences were bagged with a teabag to avoid any pollen transfer mediated by pollinators; and c) out-cross pollination: flowers were hand-pollinated with a mixture of pollen from neighbouring conspecific plants. In total, we selected 151 *Hedysarum* plants and marked 453 flowers. Approximately one month after the treatment, we collected ripe fruits, and counted the number of seeds. Reproductive success was calculated as fruit and seed production per flower. We calculated a pollen limitation index as in Tscheulin and Petanidou (2013):

$$1 - \frac{\text{seed set out} - \text{cross pollination treatment}}{\text{seed set open pollination treatment}}$$

Differences in fruit production between native and introduced areas and between treatments were tested by Chi-square analysis. Differences in pollen limitation were tested by a linear mixed

model with area (native/introduced) as fixed effect and plot as random effect.

## 3. Results

### 3.1. Pollinator identity and degree of generalization of *Hedysarum*

We conducted a total of 248 censuses ranging from 7 (105 min) to 29 (435 min) censuses per plot; 112 were conducted in the native area (43 and 69 in 2009 and 2010, respectively) and 136 in the introduced area (34 and 102 in 2009 and 2010, respectively). During these censuses we observed 6653 visits achieved by 21 pollinator species in the native area and 20 in the introduced area, all of which were observed to legitimately visit *Hedysarum*. No nectar robbery was detected. The native area shared seven species with the introduced area (Appendix C).

The species belonged to 20 different genera of 11 families and three orders: Coleoptera, Diptera and Hymenoptera, with Hymenoptera being by far the most represented order (79.41%) with 27 species. We sporadically observed lepidopterans but we did not consider them because during their visits they did not touch the reproductive parts of the flowers.

The shared species were five hymenopterans and two coleopterans, representing similar percentages of the pollinator species pool in the native (33.33%) and in the introduced (35.00%) area and achieving the 93.69% and the 85.76% of the visits in each area, respectively. Dipterans were detected only in the native area.

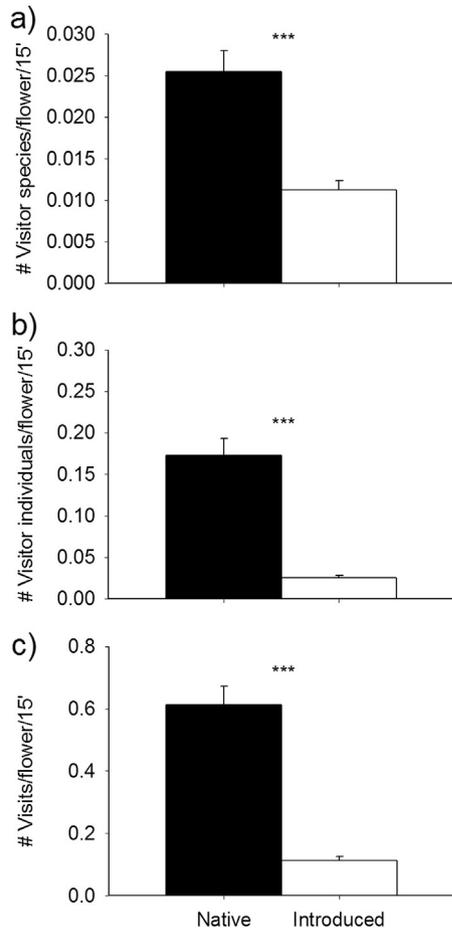
In 2009, the expected degree of generalization (i.e. according to the first-order Jackknife; mean  $\pm$  95% confidence interval are shown) of *Hedysarum* was higher in the native area ( $19.84 \pm 2.58$  visitor species) than in the introduced area ( $9.91 \pm 1.68$ ). However, in 2010 the opposite trend was found with a lower number of pollinator species visiting *Hedysarum* in the native ( $18.94 \pm 1.97$ ) than in the introduced ( $30.89 \pm 3.43$ ) area. With both years pooled, differences between areas were not significant (native:  $30.92 \pm 3.29$ , introduced:  $31.92 \pm 3.29$ ).

### 3.2. Pollinator richness, abundance, and visitation rates

Pollinator richness ( $n = 248$ ,  $df = 4$ ,  $Z = -5.38$ ,  $P < 0.001$ ), abundance ( $n = 248$ ,  $df = 4$ ,  $Z = -7.05$ ,  $P < 0.001$ ), and visitation rates ( $n = 248$ ,  $df = 4$ ,  $Z = -8.88$ ,  $P < 0.001$ ) were higher in the native than in the introduced area (Fig. 2). For all variables, year explained much of the variance, pointing to a high inter-annual variability in pollinator assemblages. In fact, the general trend of higher pollinator richness, abundance, and visitation rates in the native area was found mainly in 2010.

The higher visitation rates in the native area were due to the higher pollinator abundance, as the number of visits per individual pollinator did not differ between study areas (native =  $4.68 \pm 0.33$ , introduced =  $5.76 \pm 0.56$ ,  $n = 206$ ,  $t = 1.48$ ,  $df = 156.77$ ,  $P = 0.14$ ).

The honeybee, appearing in 169 (92 and 77 in native and introduced areas, respectively) out of the 248 censuses (68.15%) and being present in both areas, was the most abundant pollinator and the one that made the most visits both in the native and in the introduced areas during the two study years (Table 1). The rest of pollinator species showed values largely below the ones for the honeybee, appearing in the  $1.78 \pm 0.39\%$  of the censuses and only contributing to the  $0.28 \pm 0.11\%$  of the total visits. Moreover, the contribution of the honeybee to *Hedysarum* pollination matched the trend found for the total pollinator pool in terms of abundance (native =  $0.18 \pm 0.02$ , introduced =  $0.03 \pm 0.01$ ,  $Z = -5.26$ ,  $df = 4$ ,  $P < 0.001$ ) and visitation rate (native =  $0.68 \pm 0.06$ , introduced =  $0.15 \pm 0.02$ ,  $Z = -6.00$ ,  $df = 4$ ,  $P < 0.001$ ), which were also significantly higher in the native than in the introduced area



**Fig. 2.** Mean ( $\pm$ SE) for richness (a), abundance (b) and visitation rates (c) of *Hedysarum* pollinators in the native (filled bars) and introduced (open bars) areas pooled for the two sampling years.

and mainly determined by 2010 data. Meanwhile, the number of visits achieved per honeybee individual did not differ between areas (native =  $5.39 \pm 0.14$ , introduced =  $6.85 \pm 0.45$ ,  $t = 1.58$ ,  $df = 167$ ,  $P = 0.12$ ).

### 3.3. Honeybee pollen loads

*Hedysarum* pollen grains represented high and not significantly different percentages of the pollen loads on honeybees in both native ( $99.335 \pm 0.002\%$ ) and introduced ( $99.183 \pm 0.002\%$ ) areas ( $n = 198$ ,  $F = 1.83$ ,  $df = 6$ ,  $P = 0.23$ ), as well as in both ventral ( $99.383 \pm 0.002\%$ ) and dorsal ( $99.106 \pm 0.002\%$ ) parts of their bodies ( $n = 198$ ,  $F = 2.07$ ,  $df = 97$ ,  $P = 0.15$ ). The interaction of these factors was not significant, either ( $n = 198$ ,  $F = 0.15$ ,  $df = 97$ ,  $P = 0.70$ ).

**Table 1**

Total number and percentage (mean  $\pm$  SE) of individuals and visits of the honeybee to *Hedysarum* in native and introduced areas.

Area	Year	n	# Honeybee individuals	% Honeybee individuals	# Honeybee visits	% Honeybee visits
Native	2009	43	75	$76.48 \pm 2.31$	511	$89.48 \pm 1.84$
	2010	69	965	$87.47 \pm 1.12$	3733	$91.95 \pm 0.79$
Introduced	2009	34	101	$77.27 \pm 3.69$	645	$93.00 \pm 1.67$
	2010	102	165	$69.78 \pm 2.34$	1066	$80.79 \pm 2.03$

### 3.4. Pollen loads on stigmas

Of the 119 stigmas analysed, only five had no pollen grains adhering (two collected in the native area and three in the introduced area). In the remaining 114 stigmas *Hedysarum* dominated pollen loads. In 111 cases (97.37%), only *Hedysarum* grains were found, while in the other three, heterospecific pollen was just present (i.e. one grain) or in low quantity (<25%). The frequency of the observed pollen loads did not vary between native and introduced areas (Chi-square test,  $P > 0.1$  in all cases) (Table 2).

### 3.5. Fruit and seed sets

None of the bagged flowers (i.e. pollinator exclusion) produced fruits autonomously, either in the native or in the introduced area. Fruit production of the other two treatments pooled together was higher in the native than in the introduced area (Chi-square = 29.28,  $df = 1$ ,  $P < 0.001$ ). In the native area, fruit production in open pollinated flowers did not differ from out-cross pollinated flowers (Chi-square = 2.02,  $df = 1$ ,  $P = 0.16$ ). However, in the introduced area out-cross pollinated flowers set more fruits than did open pollinated flowers (Chi-square = 4.10,  $df = 1$ ,  $P = 0.04$ ).

We found a similar pattern for pollen limitation index, which was higher in the introduced area ( $n = 82$ ,  $t = 2.79$ ,  $df = 6$ ,  $P = 0.03$ ) (Fig. 3). Only positive values were included in the analyses as, according to Larson and Barrett (2000), negative values might result from experimental or type I statistical error.

## 4. Discussion

The degree of generalization of *Hedysarum* populations did not differ between the native and introduced areas even when only 20.59% of the pollinator species were shared between the two areas. The presence of shared pollinators was not due to introduced pollinators establishing alien complexes (*sensu* Olesen et al., 2002) since all of them were native in both areas. Stout et al. (2006) found a similar pattern for *R. ponticum*, as invasive populations of this species in Ireland showed similar generalization levels compared to native populations in southern Spain, though pollinator identities differed. Despite the high level of generalization, in both areas the honeybee was the most common pollinator of *Hedysarum* and the one that made most of the visits, as found in other areas (Satta et al., 2000; Galloni et al., 2008). This finding points to the important role that domestic social pollinators play in the integration of non-native plants into new regions (Parker and Haubensak, 2002; Grabas and Laverty, 1999; Barthell et al., 2001; Jesse et al., 2006). In *Hedysarum*, native and introduced areas belong to the same biogeographical region, and both are included in the native distribution range of the honeybee (Goulson, 2003). The honeybee is a super-generalist pollinator (Huryn, 1997) that can broaden its diet to include new food resources, including non-native plants (Stout et al., 2002; Simpson et al., 2005; Gross et al., 2010). Moreover, it has been widely introduced around the world being also well integrated (Moritz et al., 2005). Therefore, for plants pollinated by the honeybee, finding suitable pollinators when introduced in a new area might be more common than it was previously expected (e.g. *C. scoparius*; Simpson et al., 2005).

Despite that *Hedysarum* was well integrated into the diet of resident pollinators in the introduced area, its reproductive success in terms of fruit set proved lower than in the native range. Differences were not due to contrasting reproductive strategies among study areas but rather to pollen limitation. Pollen limitation can result from reduced quantity and/or quality of pollen deposited on stigmas (Aizen and Harder, 2007).

**Table 2**  
Number of *Hedysarum* stigmas in the native ( $n = 65$ ) and introduced ( $n = 51$ ) areas carrying different percentages of conspecific and heterospecific pollen grains classified in the following categories: absent, present (only one grain), low ( $\leq 25\%$ ), medium ( $25\% < > 75\%$ ), high ( $\geq 75\%$ ) or sole. Chi-square statistics and  $P$ -values of the contingency tables for each category and type of pollen are given.

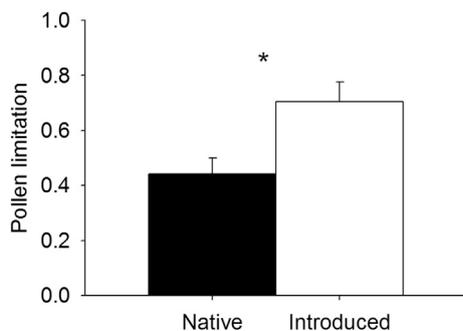
	Conspecific				Heterospecific			
	$n$ Native	$n$ Introduced	Chi-square	$P$	$n$ Native	$n$ Introduced	Chi-square	$P$
Absent	2	3	0.05	0.83	65	51	1.79	0.18
Present	0	0	–	–	0	2	0.72	0.40
Low	0	0	–	–	0	1	0.01	0.93
Medium	0	0	–	–	0	0	–	–
High	0	3	1.79	0.18	0	0	–	–
Sole	63	48	1.89	0.17	0	0	–	–

We could not estimate whether the total quantity of pollen depositions on stigmas was lower in the introduced area than in the native area because our methodology was semi-quantitative (i.e. we explored relative abundances of pollen depositions, but not absolute values). However, pollen transfer is a function of visitation rate (Wilcock and Neiland, 2002), which in many cases is a good predictor of reproductive success (Vázquez et al., 2005; Dauber et al., 2010; but see Dietzsch et al., 2011) and in the introduced area visitation rate was lower than in the native area. The lower visitation rate might be a direct consequence of the lower pollinator abundance found in the introduced area. The lower pollinator abundance in introduced *Hedysarum* populations could have various non-mutually exclusive explanations. First, resident pollinators could not be intensively exploiting this resource yet (Armbruster and Herzig, 1984). That is, despite resident pollinators including *Hedysarum* in their diets, and in fact introduced populations showed similar generalization level (i.e. total number of pollinator species) as native ones, they visited *Hedysarum* less frequently, as indicated by the lower richness (i.e. pollinator species per flower and observation period). If floral resources are locally abundant, introduced plant species might compete with natives for pollinators. Natives with higher relative flower abundance than *Hedysarum* could be attracting more pollinator visits (Rathcke, 1988; Burns et al., 2011; Dietzsch et al., 2011). Despite that *Hedysarum* dominated plant communities and had similar cover in both study areas, some native plants exceeded its floral offer (i.e. *Calicotome infesta* and *Lotus cytisoides*, unpublished data) in the introduced area. Moreover, irrespective of relative plant abundances, pollinators might still prefer feeding on native floral resources they are used to, as far as they cover their feeding needs, showing a lag time (Crooks, 2005) to achieve the same pollination levels to *Hedysarum* as in the native range.

Second, baseline pollinator abundance could be lower in the introduced area than in the native area, moreover being the introduced area an island and being the main pollinator a managed

species. However, our analysis of three plant species present and native to the two study areas, and that share pollinators with *Hedysarum*, did not suggest that pollinators with potential to visit *Hedysarum* may be less abundant in the introduced compared to the native area as analysed species showed similar pollinator richness, abundance, and visitation rates in both areas (Appendix D). The impoverished biota in comparison with corresponding continental areas typifies oceanic islands (Wardle, 2002) and Menorca is a continental island that was connected to mainland during the Messinian period (between 5.70 and 5.35 million years ago) (Alcover, 2010). In addition, in this study case a single pollinator species is the responsible of more than the 80% of the visits in both areas, so that we would not expect insularity to be affecting the results. Nevertheless, we cannot disregard the fact that the study introduced area is an insular ecosystem where pollinator fauna might be depauperated (MacArthur and Wilson, 1967). We can neither rule out the possibility that the different honeybee abundances found between study areas were due to differences in management and not to the species integration into resident plant–pollinator communities. Even baseline pollinator abundance been similar in native and introduced areas, pollinator abundance in the introduced area could be lower at the local scale of study (i.e. 400 m<sup>2</sup> plots), for instance due to lower local flower abundance. Unfortunately, we lack such detailed information for the native area to corroborate or refute this explanation.

Regarding the quality component of pollen limitation, in our study case it seems to be less influential than the quantitative one. This quality component embraces many aspects that include not only the presence of heterospecific pollen, but also other aspects not directly analysed in this study such as the genetic relatedness of the donor (Souto et al., 2002), or the pollinators effectiveness in terms of matching between their phenology and body size with receptive plant stigmas (Nienhuis and Stout, 2009). However, we would not expect the pollen limitation observed in introduced populations to be due to a shift in pollinator identity (Larson et al., 2002; Bartomeus and Vilà, 2009) and subsequent differences in pollinator effectiveness (Lau and Galloway, 2004; Dohzono and Yokoyama, 2010; Ne'eman et al., 2010) as in both areas the honeybee made more than the 80% of the visits and the other pollinator species on average only achieved around the 0.30% of the visits. In addition, the honeybee is an effective pollinator of *Hedysarum* (Satta et al., 2000) and its foraging behaviour appeared to be the same in both areas, making a similar number of visits per individual per patch and carrying similarly high percentages of *Hedysarum* pollen loads. Nevertheless, even with the same principal pollinator species showing the same foraging behaviour in native and introduced areas, we cannot dismiss the possibility of lower quality pollen reaching *Hedysarum* stigmas in the introduced area. Introduced populations are usually less genetically diverse due to founder effects (Barrett and Husband, 1990). Despite we lack information about the genetic structure of studied populations to



**Fig. 3.** Mean (+SE) pollen limitation index in native (filled bar) and introduced (open bar) areas.

corroborate this hypothesis, *Hedysarum* individuals in introduced populations could thus be more closely related and the same pollen transfer patterns could induce higher inbreeding rates in the area of introduction.

Overall, we cannot infer the demographic consequences of the higher pollen limitation observed in the introduced area (Feinsinger, 1987). The establishment and spread of non-native species are long-term processes that do not depend on the success of a single reproductive season (e.g. Downey and Brown, 2000). In short-lived perennials such as our study species, one-year seed production might be more related to population demography (Parker, 1997) than in long-lived non-native species. However, the lower seed set of non-native species can be counteracted by other biotic and abiotic factors acting in other stages of the plant–life cycle (Blackburn et al., 2011; Carrillo-Gavilán et al., 2012). Lloret et al. (2005), for instance, found that for 350 naturalized plant species across the Mediterranean region, seed dispersal correlated better with non-native species abundance than did pollination. Moreover, constant propagule pressure of *Hedysarum* annual seeding can also counterbalance pollinator and pollen deficiencies.

In order to extrapolate these results to other plant species or even to *Hedysarum* in other introduced areas, some considerations should be made. First of all, studied introduced populations are established and spreading (according to Blackburn et al., 2011) and influential factors differ over the different stages of the naturalization and eventual invasion process (Lloret et al., 2005; Aizen et al., 2008). Second, study native and introduced areas belong to the same biogeographical region and results might differ from cases in which plants are introduced in completely distant and dissimilar biogeographical regions. Further research applying this biogeographical approach is needed in other case studies to overcome these limitations and reach stronger generalizations.

## 5. Conclusion

This study adds evidence to the integration of non-native plants into resident plant–pollinator networks reported in other systems. However, and contrary to our hypothesis, our biogeographical approach has shown that such integration does not prevent pollen limitation in the introduced area. Therefore, integration of non-native plants into the native plant–pollinator community, despite being necessary, might not be the key for their persistence and spread in introduced areas.

## Author contributions

AMC and MV conceived and designed the experiments. AMC and MV conducted the field work. FJOS classified the insects. AMC performed the statistical analyses. AMC, MV and FJOS wrote the manuscript.

## Acknowledgements

We are grateful to C. Rodríguez-Rodríguez for statistical advice and D. Nesbitt and J. Macaluso for English revision. We thank J.J. López-Pérez and M.A. Marcos for Coleoptera and Diptera identifications, respectively. Baelo Claudia Archaeological Ensemble (Cádiz) and Albufera des Grau Natural Park (Menorca) for allowing us to work in their precincts. A. Barón, R. Ruiz, E. Manzano, M. Vallès, V. Sánchez, R. Molina and M. López helped with the field work. EBD receives financial support from the Spanish Ministerio de Economía y Competitividad, through the Severo Ochoa Program for Centres of Excellence in R+D+I (SEV-2012-0262). Funding was provided by the Spanish Ministry of Ciencia e Innovación project

REDESIN (CGL 2007-61165-BOS) and by the EU 7FP project STEP (244090-STEP-CP-FP). AMC was supported by a JAE-Predoc fellowship.

## Appendix A. Supplementary material

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.actao.2014.01.001>.

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